Mechanism of the Chemiluminescence of Lucigenin. II. The Charge-transfer Structure of Lucigenin and Reduction of 10,10′-Dimethyl-9,9′-biacridinium Dication by Electron Transfer from Nucleophiles

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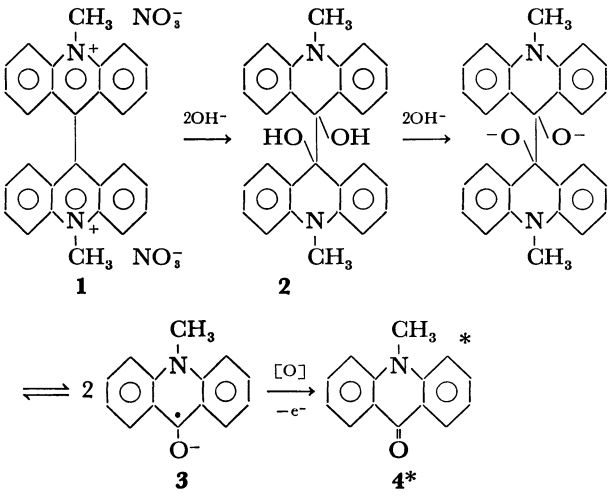
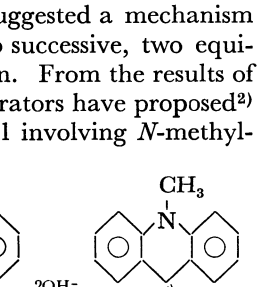
Lucigenin (10,10′-dimethyl-9,9′-biacridinium dinitrate, $DBA^{2+}2NO_3^-$) was found to be a CT complex between DBA^{2+} and NO_3^-. In the reaction of lucigenin with several nucleophiles in the absence of molecular oxygen or oxidizing agents the following four types of products were obtained, depending upon the relative power of the nucleophiles to donate electrons to DBA^{2+}: (1) salts of DBA^{2+} ($DBA^{2+}2X^-$) which are the CT complex between DBA^{2+} and the nucleophiles, such as Cl^-, Br^-, SCN^-, and I^-, (2) the cation radical $DBA^{+\cdot}$ produced by one-electron transfer from nucleophiles, such as $C_6H_5COCH_2^-$ and $CH_3COCH_2^-$, to DBA^{2+}, (3) the biradical $DBA^{\cdot}_2$ produced by two-electron transfer from nucleophiles such as OH^-, CN^-, CCl_3^-, and $C_6H_5S^-$ and (4) 10,10′-dimethyl-9,9′-bi(dihydroacridinylidene) produced from $DBA^{\cdot}_2$. Only the nucleophiles of (3), which produced $DBA^{\cdot}_2$ brought about the luminescence of lucigenin in the presence of oxygen, and $DBA^{\cdot}_2$ also showed luminescence in organic solvents in the presence of oxygen. From these findings it was concluded that the first process of the chemiluminescent reaction of lucigenin was the electron transfer reduction of DBA^{2+} by the nucleophiles to form the biradical $DBA^{\cdot}_2$.

Numerous studies concerning the mechanism of the chemiluminescenec of lucigenin($DBA^{2+}2NO_3^-$, **1**) with hydrogen peroxide in an aqueous alkaline solution have been carried out.[1] It has been established that *N*-methylacridone, the end product of the luminescence, is a light emitter.[1g] Totter has suggested a mechanism for the luminescence involving two successive, two equivalent reductions and autoxidation. From the results of an ESR study Janzen and collaborators have proposed[2] the mechanism shown in Scheme 1 involving *N*-methylacridone ketyl, **3**. Maeda and Hayashi have previously reported that crystals of **1** exhibited a weak ESR absorption,[3a] and Maeda, Hayamizu, and Hayashi have shown that an aqueous sodium hydroxide solution of **1** produced a compound[3b] with the same UV and IR spectra as 10,10′-dimethyl-9,9′-bi(dihydroacridinylidene), **5**, but in contrast to **5**, the product exhibited an ESR absorption both in solid state and in solution and luminescence with molecular oxygen in solutions of organic solvents. These findings suggest a radical mechanism of the luminescence involving a reduction process of **1** by a hydroxide ion. In order to obtain further information on the radical mechanism of the chemilumnescence of **1**, its reactions with the various nucleophiles shown in Table 1 were studied. This paper deals with the results of the reactions and discusses the mehanism involving a reduction process of DBA^{2+}.



Scheme 1.



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Experimental

Measurements. Measurements of electronic absorption spectra were carried out using a Shimadzu UV 200 spectrophotometer with oxygen-free solutions which were purged with prepurified nitrogen. The IR spectra were measured on a JASCO IR-G infrared spectrophotometer. The ESR spectra and spin concentrations were obtained by a JES p-10 type ESR spectrometer (100 kHz modulation) equipped with a JES 1D-2 type integrator with deaerated solutions and solids. The proton NMR spectra were measured with a JEM C-60-HL NMR spectrometer. The fluorescence spectra were obtained with a Hitachi EPF-2A fluorescence spectrophotometer with deaerated solutions.

Materials. Lucigenin, **1**, was synthesized by the method of Decker and Dunnant[4] from *N*-methylacridone which was obtained from acridine. Purification of **1** was achieved by recrystallization from water and then ethanol to give amber crystals. NMR(D_2O); δ 5.07(6H, s, N–CH_3), δ 7.26—8.52 (16H, m, aryl proton). 10,10′-Dimethyl-9,9′-bi(dihydroacridinylidene), **5**, was prepared by the reduction of *N*-methylacridone with zinc and acetic acid, and recrystallized from pyridine to give lemon yellow fine crystals.

Results and Discussion

The addition of aqueous solutions containing the nucleophiles of group (a) in Table 1 to an aqueous

Table 1. The chemiluminescence of an aqueous solution of **1** ($DBA^{2+}2NO_3^-$) upon the addition of aqueous solutions of nucleophiles in the presence of oxygen

Nucleophile (a)	Chemi-luminescence	Nucleophile (b)	Chemi-luminescence
$(C_2H_5)_3N$	+	Cl^-	−
NH_3	+	Cr^-	−
$NH_2NH_2H_2O$	+	SCN^-	−
$CH_3COCH_2^-$	+	I^-	−
$CH_3SOCH_2^-$	+		
CCl_3^-	+		
OH^-	+		
t-BuO^-	+		
CN^-	+		
S^{2-}	+		
$C_6H_5S^-$	+		

+: Chemiluminescent; −: Non-chemiluminescent
Concentration of **1**: 1×10^{-4} mol l^{-1}.

solution of **1** in the presence of oxygen brought about the luminescence of **1**, but the addition of the nucleophiles of group (b) in Table 1 under the same conditions did not result in any luminescence. On the other hand, a DMF solution of **1** exhibited luminescnce in the presence of oxygen with or without the nucleophiles of group (b) in Table 1. In order to obtain some information about the difference in behavior between the nucleophiles of group (a) and (b) of Table 1 for the chemiluminescence of **1**, reactions of these nucleophiles with **1** were studied in oxygen-free solutions in which no luminescence was exhibited. It was found that the following four types of products, $DBA^{2+}2X^-$, DBA^+X^-, $DBA^{\cdot}$, and DBA, **5**, were produced, depending upon the kind of nucleophiles and reaction conditions, as described below.

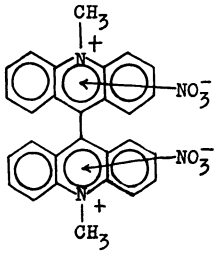
I. Salts of 10,10'-Dimethyl-9,9'-biacridinium Dication, DBA^{2+} 2X⁻. When solids of potassium or sodium salts of Cl^-, Br^-, SCN^-, and I^-, the nucleophiles of group (b) in Table 1, were added to an aqueous solution of **1** (1×10^{-2} mol l^{-1}) in amounts equivalent to twice the molar quantity of **1**, salts of DBA^{2+} with the nucleophilic anions added, $DBA^{2+}2Cl^-$(**6**), $DBA^{2+}2Br^-$(**7**), DBA^{2+} $2SCN^-$(**8**), and DBA^{2+} $2I^-$(**9**), as by elemental analysis of Cl, Br, S, and I, were separated out. The colors of salts **1**, **6**, and **7** were amber, while the colors of salts **8** and **9** were orange and dark red, respectively. The deepened colors of salts **8** and **9** caused by the replacement of NO_3^- by SCN^- or I^-, which has a larger nucleophilicity[5] than NO_3^-, suggest the formation of a CT complex between DBA^{2+} and SCN^- or I^-, as has been reported for the salts of pyridinium,[6] tropylium[7] and quinolinium[8] cations.

The crystals of **1** exhibited weak ESR absorption(g= 2.003, the line width was 12.5 G) and those of **6**, **7**, **8**, and **9** also exhibited weak ESR absorption(g=2.003). The spin concentrations of **1**, **8**, and **9** are shown in Table 2. The intensities of these signals increased two or three times upon irradiation with an incandescent lamp for about 1 h and reverted to their original intensities after standing in the dark at room temperature. These findings suggest that lucigenin is also

Table 2. The spin concentrations and colors of the CT complexes $DBA^{2+}2X^-$ measured in the absence of the light

X^-	Spin mol^{-1}	Color
NO_3^-	7×10^{18}	amber
SCN^-	1.5×10^{19}	orange
I^-	1×10^{20}	dark red

the CT complex **1'** shown below, although the degree of charge transfer was less than those in **8** and **9**.



1'

Confirmation of the CT structure of these salts was carried out by measurements of their absorption spectra. For example, the absorption spectra of **9** measured in mixed solvents of DMSO, $CHCl_3$, and CH_2Cl_2 showed a broad band in a longer wavelength region than 500 nm, which was not observed in water, as shown in Fig. 1. The band was attributed to charge transfer from I^- to DBA^{2+}, since the absorbance of the band was intensified for a decrease in the polarity of the mixed solvent and disappeared in water. This fact appears to indicate a shift of equilibrium in solutions, shown in Scheme 2, which favors an ionic species in polar solvents. Comparison of the CT absorption of **1**, **6**, **7**, **8**, and **9** measured in ethanol, DMSO, and DMF,

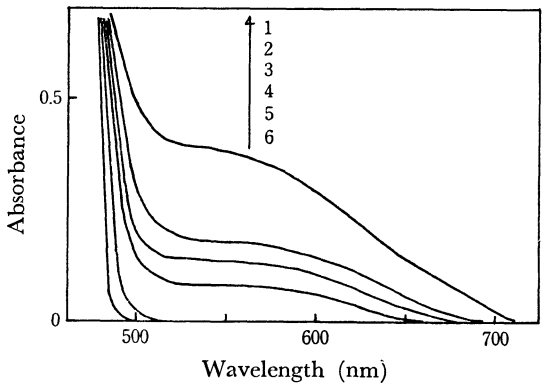
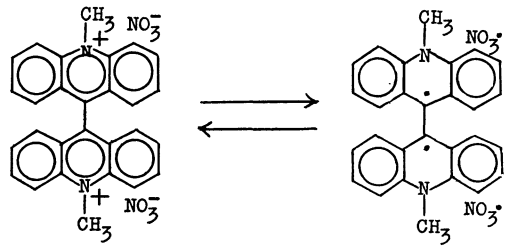


Fig. 1. The variation of the absorption spectrum of **9** with decreasing the polarity of solvents.

	Solvents (vol%)			
	CH_2Cl_2	$CHCl_3$	DMSO	H_2O
1	50	20	30	0
2	50	15	35	0
3	50	10	40	0
4	50	5	45	0
5	0	0	100	0
6	0	0	0	100

Concentration of **9**: 2.0×10^{-4} mol l^{-1}.

←increase of the polarity—



Scheme 2.

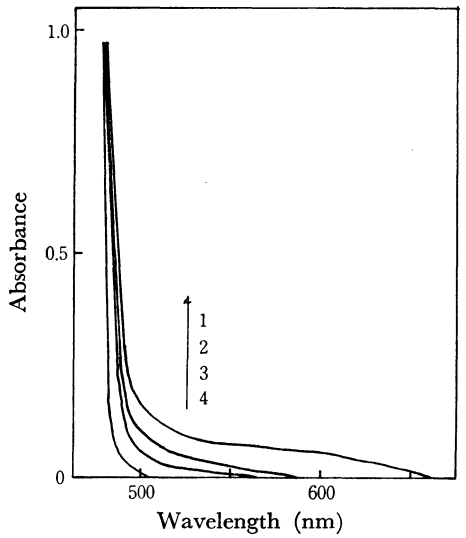


Fig. 2. The absorption spectra of $DBA^{2+}2X^-$.

	X^-		Solvent
1	I^-	**9**	DMF
2	SCN^-	**8**	DMF
3	NO_3^-	**1**	DMF
4	NO_3^-	**1**	H_2O

Concentration of $DBA^{2+}2X^-$: 1.0×10^{-3} mol l^{-1}.

respectively, showed that the degree of charge transfer from the nucleophiles to DBA^{2+} increased in the following order, $NO_3^-<Cl^-\approx Br^-<SCN^-<I^-$ and for a decrease in the Z value[9] of the solvents, $H_2O<$ethanol$<$DMSO$<$DMF. The spectra of the salts of **1**, **8**, and **9** observed in DMF are shown in Fig. 2.

The formation of similar CT complexes of DBA^{2+} was also observed in oxygen-free solutions in reactions of **1** with the nucleophiles of group (a) in Table 1. By the evaporation of a solvent immediately after adding to an aqueous solution of **1** (7.8×10^{-3} mol l^{-1}) the nucleophiles in amounts equivalent to twice the molar quantity of **1**, crystals of various CT complexes of DBA^{2+} containing the added nucleophiles were obtained. After treatment of the crystals with benzene to remove the small quantity of a by-porduct, a reduction product which appeared later, the CT complexes were purified by recrystallization from water and then from ethanol. The absorption spectra of these complexes measured in water agreed with those of **1**, **8**, and **9** in the shorter wavelength region below 500 nm, but showed an additional weak broad band in the longer wavelength region above 500 nm. The broad band is considered to be due to charge transfer between nucleophiles and DBA^{2+}, because the absorbance of

Tabte 3. The spin concentrations and colors of the CT complexes obtained from **1** and nucleophiles

Nucleophile	Spin mol^{-1}	Color	Type of the fine structure of the ESR spectrum in Fig. 3
$(C_2H_5)_3N$	1×10^{20}	brown	B
$CH_3COCH_2^-$	2×10^{20}	brown	B
CCl_3^-	4×10^{20}	brown	B
$C_6H_5COCH_2^-$	4×10^{20}	bluish brown	B
NH_3	6×10^{20}	reddish brown	B
$C_6H_5S^-$	7×10^{20}	reddish brown	A
CN^-	9×10^{20}	orange brown	A
OH^-	4×10^{21}	reddish brown	A
t-BuO^-	2×10^{22}	reddish brown	A

the band measured in water was intensified in DMF. These complexes exhibited strong ESR absorptions (g=2.003) both in solid state (line widths were about 11.4 G) and in oxygen-free solutions of DMF and C_6H_6. The colors and spin concentrations of the ESR abosrption in solid state are shown in Table 3. The intensities of the ESR signals increased upon irradiation and reverted to the original intensities after standing in the dark at room temperature. These properties of the complexes are comparable to those of **1**, **8**, and **9**, although the degrees of charge transfer from the nucleophiles of group (a) in Table 1 to DBA^{2+} are larger than those from SCN^- and I^- (the nucleophiles of group (b) in Table 1) and NO_3^-. ESR spectra of the CT complexes containing the nucleophiles of group (a) in Table 1 measured in oxygen-free solutions showed fine structures which are classified into two groups, A and B, shown in Fig. 3. Although it is now difficult to explain the two types of the fine structures, it is observed that (1) the CT complexes which exhibited a spectrum of type A (the spacing was 1.6 G) had spin concentrations larger than those of the CT complexes which exhibited a spectrum of type B (the spacing was 3.0 G) as shown in Table 3 and (2) fine structure of type A is similar to that of the ESR spectrum[2a] which was attributed to the radical cation of DBA^{2+} produced by the one-electron reduction of DBA^{2+}. From these findings it is conceivable that both in solid state and in less polar solvents, the equilibrium

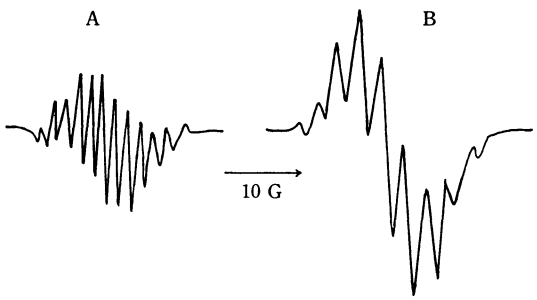
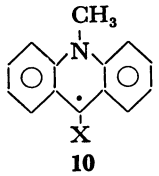


Fig. 3. Two types of ESR spectra of $DBA^{2+}2X^-$ in the oxygen-free DMF solution.
X^- showing type A: $C_6H_5S^-$, CN^-, OH^-, t-BuO^-.
X^- showing type B: $(C_2H_5)_3N$, $CH_3COCH_2^-$, CCl_3^-, $C_6H_5COCH_2^-$, NH_3.

shown in Scheme 2 might be further shifted more to the right-hand side in CT complexes having a fine structure of type A than in CT complexes having a fine structure of type B. Counter anions in CT complexes having a structure of type A, $C_6H_5S^-$, CN^-, OH^-, and *t*-BuO^-, might have a larger electron-donating power than the neutral or anionic nucleophiles in CT complexes having a structure of type B, $(C_2H_5)_3N$, $CH_3COCH_2^-$, CCl_3^-, $C_6H_5COCH_2^-$, and NH_3.

II. 10,10'-Dimethyl-9,9'-bi(dihydroacridinyl) Biradical DBA and 10,10'-Dimethyl-9,9'-bi(dihydroacridinylidene) Produced by Two-electron Transfer from Nucleophiles to DBA^{2+}. Although aqueous solutions of **1**, **8**, and **9** did not exhibit chemiluminescence in the presence of oxygen or hydrogen peroxide, the addition of nucleophiles of group (a) in Table 1 to these solutions produced luminescence. Luminescence of the salts was also observed in aqueous solutions containing organic solvents such as ethanol, DMSO, DMF, and pyridine in the presence of oxygen or hydrogen peroxide. It has already been reported by Janzen[2] that the addition of the potassium or sodium salts of the nucleophiles, CN^-, *t*-BuO^-, and S^{2-}, included in group (a) in Table 1, to solutions of **1** in DMSO, DMF, or aqueous DMSO in the presence of oxygen resulted in luminescence, as in the case of the addition of OH^-, and radical **10** corresponding to ketyl radical **3** was formed as an intermediate. If the mechanism involving **3** or **10** is correct, the nucleophiles



X: CN, *t*-BuO, S^-

10

of group (a) in Table 1 which exhibited luminescence in the presence of oxygen, might produce a radical corresponding to **10** in oxygen-free solutions *via* the addition at the 9,9'-positions of DBA^{2+}. This assumption was examined by measuring the variation of the absorption spectrum of **1** for the addition of the nucleophiles of group (a) in Table 1. When various amounts of the nucleophiles were added to a solution of **1** (1.6×10^{-4} mol l^{-1}) in oxygen-free aqueous ethanol (50 : 50 vol%), a new broad band appeared in the wavelength range longer than 500 nm, and the absorbance of the band increased with an increase in the nucleophile amount. Two examples are shown in Figs. 4 and 5. The broad band can reasonably be attributed to charge transfer in the salts formed between DBA^{2+} and the nucleophiles. When the nucleophiles were added to a more dilute solution of **1** (8×10^{-5} mol l^{-1}) in oxygen-free aqueous ethanol, the mixture gradually turned reddish brown and then became turbid owing to the formation of an insoluble product. The evolution of the absorption spectrum of **1** was followed in dilute solutions of **1** until turbidity appeared. No broad CT band was observed, but each spectrum showed a decrease in the absorbance of the band of DBA^{2+} at 370 nm accompanied by the increase of a band at 423 nm and two isosbestic points near 380 and 480 nm. The time-resolved absorption spectra of mixtures of

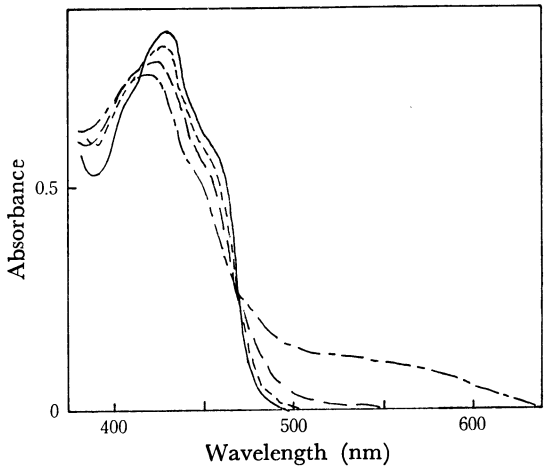


Fig. 4. The variation of the absorption spectrum of **1** with increasing the amount of $(C_2H_5)_3N$ added.
Solvent: C_2H_5OH-H_2O (50 : 50 vol%).
Concentration of **1**: 1.6×10^{-4} mol l^{-1}.
Concentration of $(C_2H_5)_3N$: —— 0, ---- 5.1×10^{-2} mol l^{-1}, — — — 1.0×10^{-1} mol l^{-1}, ——— 2.0×10^{-1} mol l^{-1}.
The spectra were measured immediately after mixing of $(C_2H_5)_3N$.

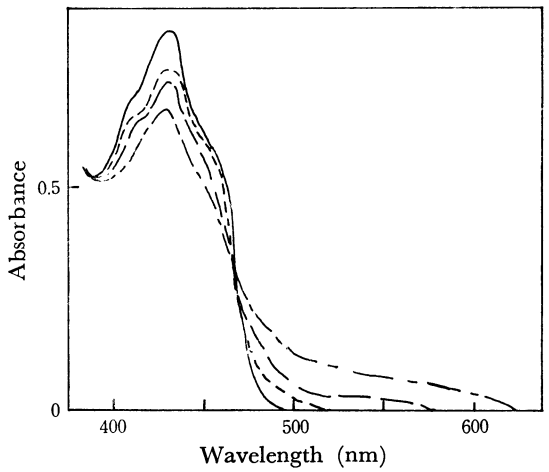


Fig. 5. The variation of the absorption spectrum of **1** with increasing the amount of *t*-BuOK added.
Solvent: C_2H_5OH-H_2O (50 : 50 vol%).
Concentration of **1**: 1.6×10^{-4} mol l^{-1}.
Concentration of *t*-BuOK: —— 0, ---- 1.1×10^{-2} mol l^{-1}, — — — 1.3×10^{-2} mol l^{-1}, ——— 1.6×10^{-2} mol l^{-1}.
The spectra were measured immediately after mixing of *t*-BuOK.

1 and $(C_2H_5)_3N$ and CN^-(NaCN) are shown in Figs. 6 and 7 as examples. A similar variation of the UV spectrum of **1** was also observed by mixing the nucleophiles in aqueous DMF and in aqueous pyridine. The variations in these solvents were faster than those in the aqueous ethanol, although they were similar. The absorption maximum at 423 nm shown in Figs. 6 and 7 agrees with that of 10,10'-dimethyl-9,9'-bi(dihydroacridinylidene), **5**. These facts and the formation of the CT complexes described above appear to show that the reaction of DBA^{2+} with the nucleophiles was the reduction of DBA^{2+} with the nucleophiles by electron

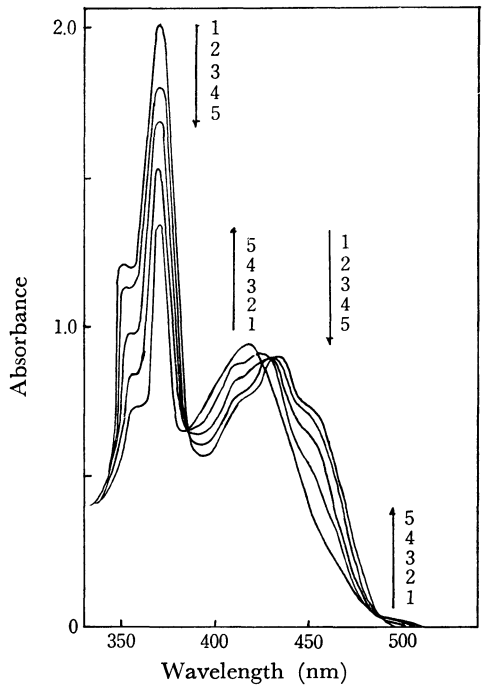


Fig. 6. The time-resolved absorption spectra of a mixture of **1** and $(C_2H_5)_3N$.
Solvent: C_2H_5OH-H_2O (50 : 50 vol%).
Concentration of **1**: 8.0×10^{-5} mol l^{-1}.
Concentration of $(C_2H_5)_3N$: 2.5×10^{-2} mol l^{-1}.
1: Before mixing, 2: 1 min after mixing, 3: 6 min after mixing, 4: 11 min after mixing, 5: 20 min after mixing.

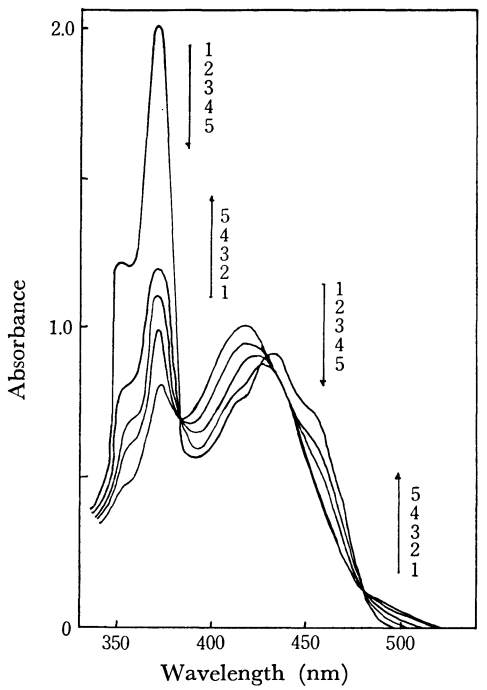
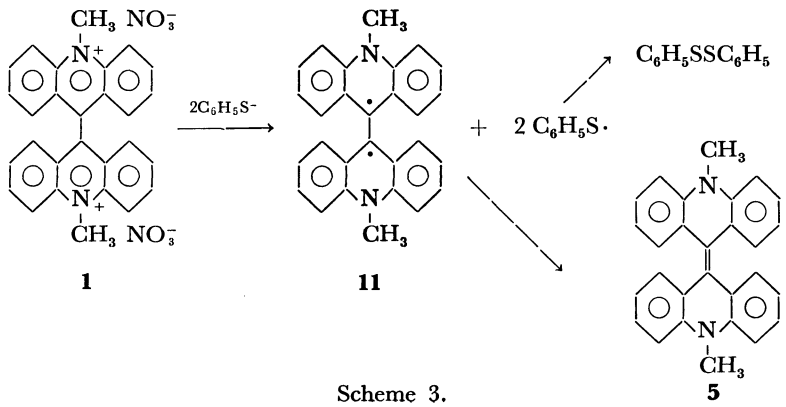


Fig. 7. The time-resolved absorption spectra of a mixture of **1** and NaCN.
Solvent: C_2H_5OH-H_2O (50 : 50 vol%).
Concentration of **1**: 8.0×10^{-5} mol l^{-1}.
Concentration of NaCN: 1.0×10^{-2} mol l^{-1}.
1: Before mixing, 2: 5 min after mixing, 3: 8 min after mixing, 4: 13 min after mixing, 5: 20 min. after mixing.

transfer *via* the formation of CT complexes rather than the formation of a radical **10** *via* the formation of pinacol **2**.[10]

The reduction of DBA^{2+} with the nucleophiles was further confirmed on the basis of the following reaction. When sodium benzenethiolate (8×10^{-4} mol) was added to an aqueous solution of **1** (4×10^{-4} mol in 45 ml) over 2 h with stirring in nitrogen, orange yellow fine crystals of the reduction product (83%) were separated out. The crystals were filtered and diphenyl disulfide (mp 60.5—61.5 °C from ethanol, 82%), the amount of which was comparable to that of the reduction product, was isolated from the filtrate. The crystals showed an absorption spectrum with a maximum at 423 nm in benzene and a green fluorescence in benzene with a maximum at 504 nm. These spectra and the IR spectrum agreed with those of **5**. The crystals decomposed at 360 °C and the results of elemental analysis[11] corresponded to **5**. However, the crystals exhibited an ESR absorption (g=2.003) in both solid state and solution. The spin concentration in solid state was 6×10^{20} spin mol^{-1}. Repeated recrystallization of the crystals from pyridine or benzene eventually gave lemon yellow crystals which exhibited no ESR absorption and were identified to be **5**. From these facts it is believed that the crystals were a mixture of **5** and a radical which is reasonably assumed to be biradical 10,10′-dimethyl-9,9′-bi(dihydroacridinyl), **11**.[12] It appears that **5** was produced from biradical **11** by bonding of the two spins and diphenyl disulfide was produced by dimerization of phenylthiyl radicals formed by one-electron oxidation of benzenethiolate



Scheme 3.

Table 4. The yields of the reduction products of DBA^{2+} obtained from **1** and nucleophiles

Nucleophile	Yield (%)
$(C_2H_5)_3N$	70
$C_6H_5S^-$	83
t-BuO^-	84
OH^-	86
S^{2-}	92
CN^-	93

anions by DBA^{2+}, as shown in Scheme 3. Because the ESR spectrum of **11** measured in C_6H_6 showed no signals attributable to a triplet state, it appears that there was little interaction between the two spins, and the two dihydroacridinyl groups in biradical **11** were twisted about the C_9–$C_{9'}$ bond. A similar reduction product of DBA^{2+} was isolated by the addition of other nucleophiles, as shown in Table 4, and it exhibited an ESR absorption which had a spin concentration of $(5—10)\times10^{20}$ spin mol^{-1}, depending upon the kind of nucleophile and the reduction condition. The spin concentration showed that the amount of biradical **11** included in the reduction product was little. Chemiluminescence was visually observed in the reduction product in organic solvents such as ethanol, benzene and pyridine in the presence of oxygen,[13] while no luminescence was observed in a pure sample of **5** under the same conditions. Therefore, when sodium dithionite ($Na_2S_2O_4$) which has a strong reducing power was used as a nucleophile, the reduction product isolated (88%) was mostly **5** and exhibited hardly any ESR absorption or chemiluminescence. Thus, it is reasonable to consider that the chemiluminescence of the reduction products is connected with the oxygenation of biradical **11**.

No reduction of DBA^{2+} in water occurred by NO_3^- and the nucleophiles of group (b) in Table 1, but occurred upon irradiation. When a dilute aqueous solution of **1** ($(5—10)\times10^{-4}$ mol l^{-1}) was irradiated with a high-pressure mercury lamp or an incandescent lamp in a nitrogen atomosphere, a solid product was gradually deposited on the wall of the reaction vessel. For example, the yield of the product was about 10% after irradiation by a 400 watt high-pressure mercury lamp for 25 h. The reduction product obtained photochemically exhibited luminescence in organic solvents in the presence of oxygen in a manner similar to the reduction products obtained by the chemical reduction of DBA^{2+} described above. The reduction of DBA^{2+} under irradiation with NO_3^- and I^- in **1** and **9** in both ethanol and DMSO was followed by observing the variation of the absorption spectra of DBA^{2+}, respectively. The variation of the spectrum of **1** in ethanol shown in Fig. 8 is similar to those shown in Figs. 6 and 7.[14] It is believed that because the electron-donating powers of nucleophiles NO_3^- and I^-(group (b) in Table 1) are weaker than those of nucleophiles of group (a) in Table 1, the former does not reduce DBA^{2+} without irradiation in water, but the latter does reduce DBA^{2+} without irradiation by the electron transfer mechanism. As described above, a DMF solution of

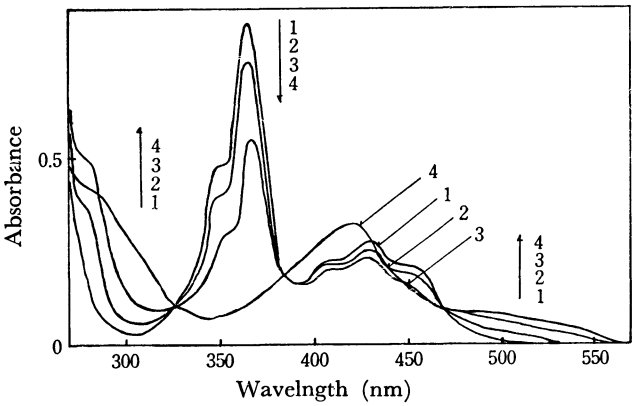


Fig. 8. The variation of the absorption spectrum of **1** in ethanol under irradiation.
Irradiation: A 400 watt high-pressure mercury lamp.
Concentration of **1**: 6.1×10^{-4} mol l^{-1}.
1: Before irradiation, 2: after irradiation for 1 min, 3: after irradiation for 3 min, 4: after irradiation for 5 min.

1 exhibited luminescence in the presence of oxygen without any other nucleophiles. This fact can be explained in terms of the electron-donating power of NO_3^- which is stronger in DMF than in water. Therefore, in the DMF solution of **1** the equilibrium of Scheme 2 was shifted greatly to the right-hand side. This explanation is further supported by the fact that a DMF solution of **1** exhibited a green fluorescence visually similar to that of **1** in water, but, in contrast to the fluorescence in water, the excitation spectrum of the fluorescence in DMF was not consistent with the absorption spectrum of DBA^{2+}, but was consistent with that of **5**, the reduction product of **1**.

III. Cation Radical DBA^+ Produced by One-electron Transfer from Nucleophiles to DBA^{2+}. It has previously been reported[3b] that an aqueous solution of **1** containing acetone turned blue upon the addition of aqueous sodium hydroxide. It was found that a similar blue color also appeared in aqueous alkaline solutions of **1** containing $RCOCH_3$ in which R was C_6H_5, C_2H_5, and H. It appears that the blue color is due to a substance produced by the reduction of DBA^{2+} with $RCOCH_2^-$ which was formed from $RCOCH_3$ and OH^-. For example, upon the addition of an ethanolic solution of acetophenone (1.74 g in 15 ml) containing aqueous sodium hydroxide (1 ml of 0.3 mol l^{-1}) to an aqueous solution of **1** (0.25 g in 45 ml) with stirring in nitrogen the solution turned blue. After 2 h, the solvent was evaporated under reduced pressure. When the residue was repeatedly extracted with water and washed with a small amount of benzene, a blue compound was obtained. The residue was soluble in benzene, pyridine, DMF, and ethanol and exhibited a strong ESR absorption in both solid state and solution (g=2.003). By evaporating the combined aqueous extracts a salt of DBA^{2+} involving $C_6H_5COCH_2^-$ as a counter ion(0.111 g) was obtained, which was recrystallized from water giving bluish-brown crystals. The salt showed an absorption spectrum having a CT absorption band in the longer wavelength range below 500 nm and showed an ESR

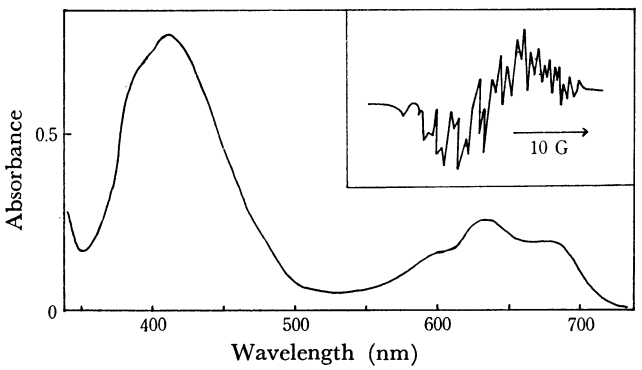
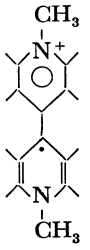


Fig. 9. The absorption spectrum of **12** in benzene and the ESR spectrum in oxygen-free DMF.

absorption both in solid state and solution. The ESR spectrum of the salt measured in an oxygen-free DMF solution was similar to the ESR spectrum of type B shown in Fig. 3.

The absorption spectrum of the blue compound **12** in benzene and the ESR spectrum in oxygen-free DMF are shown in Fig. 9. The absorption spectrum of **12** is different from that of the salt of DBA^{2+}, but resembled those of methylviologen cation radical **13**[15] and biisoquinolinium cation radical **14**,[16] which were one-electron reduction products of the parent dications, methylviologen and biisoquinolinium, respectively, although the absorption maxima of **12** (blue; 600, 630, and 680 nm in benzene) are shifted to wavelengths longer than those of **13** (royal blue; 560, 610, and 670 nm in CH_3CN[15]) and **14** (orange red; 460, 500, and 538 nm in CH_3OH[16]). Similarly to **13** which was reoxidized to the original dication by oxygen or iodine, **12** was also reoxidized to the original dication DBA^{2+} in both DMF and methanol upon the addition of an equimolar amount of iodine, because a residue obtained by evaporation of the solvents showed an absorption spectrum in water which agreed with that of DBA^{2+}. When **12** was chromatographed on silica gel using benzene as the eluent in nitrogen atmosphere, the blue color of **12** on silica gel faded and **5** was isolated as yellow crystals from the eluate. In addition, a small amount of solid which was identified to be a salt of DBA^{2+} by comparing its absorption spectrum with that of DBA^{2+}, was isolated when water was used as the second eluent. From these findings it can reasonably be assumed that the blue compound **12** is the one-electron reduction product of DBA^{2+}, DBA^{+} $C_6H_5COCH_2^-$. The cation radical corresponds to **13** and **14**, which have a common partial structure, **15**. The



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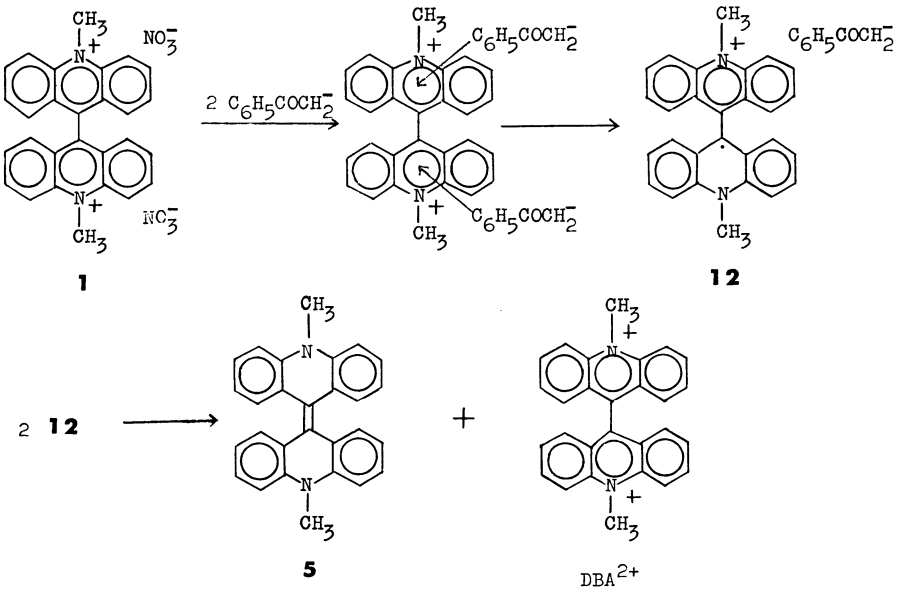
results of the elemental analysis of $DBA^{+}C_6H_5COCH_2^-$ (Found: C; 84.84, H; 5.70, N; 5.78%. Calcd for $C_{36}H_{29}N_2O$: C, 85.54, H; 5.74; N; 5.55%) and its IR spectrum, which appears to be the superposed spectra of **5** and acetophenone, also supported the structure of **12** for the blue compound. The formation of **5** and DBA^{2+} from **12** by chromatography on silica gel was considered to be due to the disproportionation reaction shown in Scheme 4. The blue color observed in the reaction of DBA^{2+} with $CH_3COCH_2^-$, C_2H_5-$COCH_2^-$, and $HCOCH_2^-$, which were formed from the corresponding ketones or acetaldehyde and hydroxide ion, was also attributed to the formation of the cation radical DBA^{+}.

The rate of formation of **12** in solutions depended upon the reaction conditions, such as the kind of solvents and the concentrations of DBA^{2+} and C_6H_5-$COCH_2^-$. When the concentrations of **1** and C_6H_5-$COCH_2^-$ were rather high, the absorption spectrum of



Scheme 4.

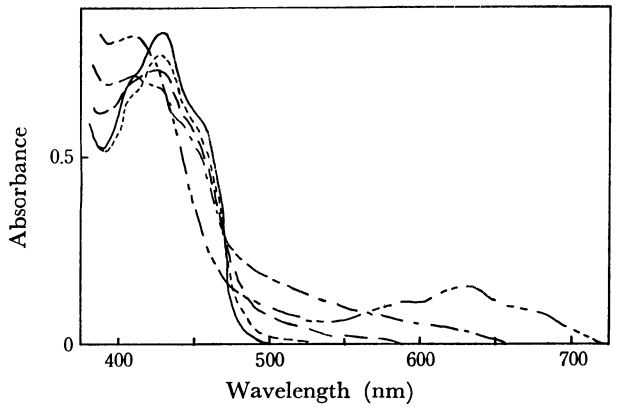


Fig. 10. The variation of the absorption spectrum of DBA^{2+} with increasing the concentration of $C_6H_5COCH_2^-$.
Solvent: C_2H_5OH-H_2O (50 : 50 vol%).
Concentration of **1**: 1.6×10^{-4} mol l^{-1}.
Concentration of $C_6H_5COCH_3$: 8.4×10^{-2} mol l^{-1}.
Concentration of NaOH added into the solution of $C_6H_5COCH_3$:
—— 0, ---- 4.7×10^{-4} mol l^{-1}, — — — 1.8×10^{-3} mol l^{-1}, ——— 2.3×10^{-3} mol l^{-1}, ——— 3.0×10^{-3} mol l^{-1}.
The spectra were measured immediately after mixing of **1** and $C_6H_5COCH_2^-$.

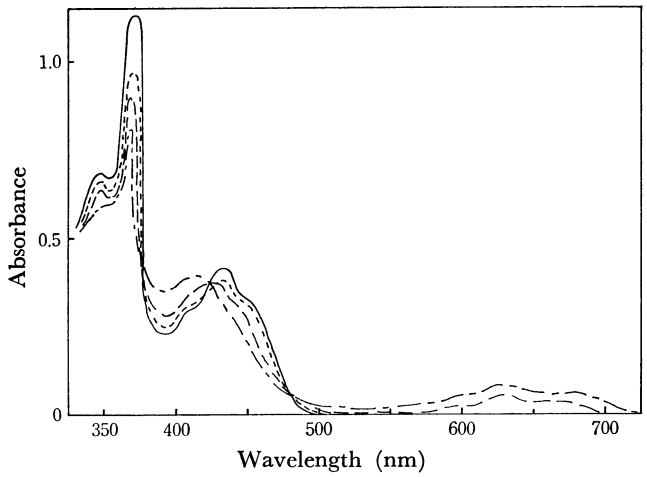
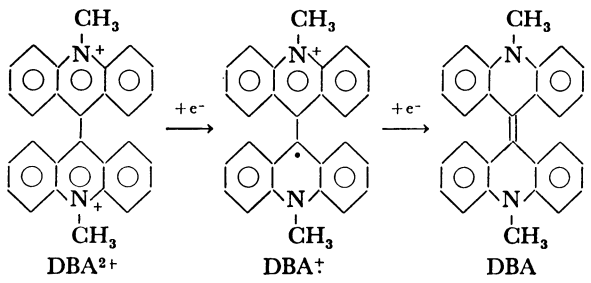


Fig. 11. The time-resolved absorption spectra of the mixture of **1** and $C_6H_5COCH_2^-$.
Solvent: C_2H_5OH-H_2O (50 : 50 vol%).
Concentration of **1**: 8.0×10^{-5} mol l^{-1}.
Concentration of $C_6H_5COCH_3$: 8.4×10^{-2} mol l^{-1}.
Concentration of NaOH added into the solution of $C_6H_5COCH_3$: 5.0×10^{-4} mol l^{-1}.
—— Before mixing, ---- immediately after mixing, — — — 10 min after mixing, ——— 40 min after mixing.

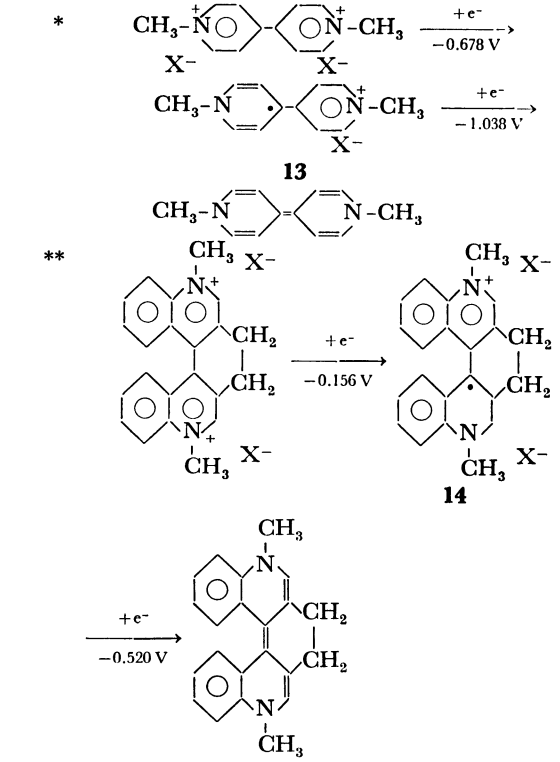
12 immediately appeared after mixing solutions of **1** and the nucleophiles, as shown Fig. 10. However, when the concentration of the nucleophiles was lower, CT absorption appeared immediately after mixing and then the absorption spectrum gradually changed to that of **12**, as is shown in Fig. 11.

In order to explain the fact that only nucleophile $RCOCH_2^-$ formed the cation radical DBA^+, one- and two-electron half-wave reduction potentials of DBA^{2+} in **1**, shown in Scheme 5, were measured polarographically[17] to be −0.035 and −0.43 V, respectively. In a manner similar to methylviologen dication*[18] and biisoquinolinium dication**,[16] the reduction potentials showed that the one-electron reduction in the first step from the dication to the corresponding cation radical **12** occurred more easily than that in the second step from the cation radical, **12**, to **5**. Although most of the oxidation-reduction potentials of the nucleophiles used in the present study are not known, it is reasonable to assume that the electron-donating power of nucleophiles $RCOCH_2^-$ to DBA^{2+} was stronger than those of NO_3^-, SCN^-, and I^- which gave CT complexes in an aqueous solution, but weaker than those of CN^-, OH^-, and CCl_3^- which gave DBA^{2+} as a reduction product. Therefore, the nucleophile $RCOCH_2^-$ formed the cation radical DBA^+ because of the difficulty in the second reduction step from DBA^+ to the two-electron reduction product.



Scheme 5.

The results obtained in I, II, and III are summarized below and the mechanism of the chemiluminescence of lucigenin, **1**, is reasonably concluded to be that described below.



1. Lucigenin($DBA^{2+}2NO_3^-$) is a CT complex between DBA^{2+} and NO_3^-. Nucleophiles, such as Cl^-, Br^-, SCN^-, and I^-, give CT complexes upon their introduction into an aqueous solution of **1**.

2. The first process of the chemiluminescent reaction of **1** in an aqueous solution in the presence of oxygen is a reduction-oxidation reaction in which DBA^{2+} is reduced by two-electron transfer from OH^- to the biradical $DBA^{\cdot}_{\cdot}$ *via* the cation radical DBA^{+}. The biradical forms dioxetane[19] by oxygenation and then produces excited *N*-methylacridone, the light emitter. The mechanism of the chemiluminescence of **1** is shown in Scheme 6.

$$\underset{\mathbf{1}}{DBA^{2+}2NO_3^-} \xrightarrow{OH^-} \underset{\text{cation radical}}{(DBA^{+})} \xrightarrow{OH^-} \underset{\mathbf{11}}{DBA^{\cdot}_{\cdot}} \xrightarrow{O_2} \text{dioxetane}$$

$$\longrightarrow \underset{\mathbf{4^*}}{N\text{-methylacridone}^*} \longrightarrow h\nu + \underset{\mathbf{4}}{N\text{-methylacridone}}$$

Scheme 6.

3. Several nucleophiles which have strong electron-donating powers, such as CN^-, *t*-BuO^-, $C_6H_5S^-$, CCl_3^- and NH_3, also give the biradical which exhibits luminescence in organic solvents in the presence of oxygen. Nucleophiles which have weak electron-donating powers, such as $C_6H_5COCH_2^-$ and $CH_3COCH_2^-$, give cation radicals by one-electron transfer *via* CT complexes, but do not produce the biradical, and consequently exhibit no luminescence in the presence of oxygen.

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10) If pinacol **2** were formed in a first process and then changed to *N*-methylacridone ketyl **3**, as shown in Scheme 1, an absorption spectrum due to pinacol **2** must appear in the wavelength region below that of **5**. In fact, the absorption spectrum of 10-methyl-9-phenyl-9,10-dihydro-9-acridinol, which is considered to have an absorption spectrum similar to that of **2**, showed only a maximum at 285 nm in ethanol.

11) Found: C, 86.72, H; 5.66, N; 7.34%. Calcd for **5**, $C_{28}H_{22}N_2$: C; 87.02; H; 5.74; N; 7.24%.

12) It appears that the biradical corresponds to a biradical which was suggested by Tamamushi and Akiyama[1e] in 1939 to be the product in the primary reaction in the proposed reaction mechanism of the chemiluminescence of **1**, on the basis of their finding, *i. e.* the formation of a brownish substance upon the warming or irradiation of a dilute alkaline solution of **1**.

13) Details of the chemilumunescence spectrum will be reported in a future publication.

14) When the photochemical reactions of **1** in both ethanol and DMSO were carried out for 2 h, a new compound which was determined to be a cyclization product of **11** was produced by further photolysis of **11** in the solutions. The new compound will be reported in a future publication.

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17) The measurement of the reduction potentials of **1** by a polarographic method was carried out by Professor Kazuo Nakada of the University of Electro-Communications. The potentials were measured *versus* a mercury electrode in DMF with tetramethylammonium iodide as the supporting electrolyte.

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